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FIELD ASSESSMENT OF YEAST- AND OXALIC ACID–GENERATED CARBON DIOXIDE FOR MOSQUITO SURVEILLANCE[1]

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ABSTRACT. Carbon dioxide (CO_2) sources improve the efficacy of mosquito traps. However, traditional CO_2 sources (dry ice or compressed gas) may be difficult to acquire for vector surveillance during military contingency operations. For this reason, a new and convenient source of CO_2 is required. Two novel CO_2 generators were evaluated in order to address this capability gap: 1) an electrolyzer that converts solid oxalic acid into CO_2 gas, and 2) CO_2 produced by yeast as it metabolizes sugar. The flow rate and CO_2 concentration produced by each generator were measured, and each generator's ability to attract mosquitoes to BG-Sentinel™ traps during day surveillance and to Centers for Disease Control and Prevention light traps with incandescent bulbs during night surveillance was compared to dry ice and compressed gas in Jacksonville, FL. The electrolyzed oxalic acid only slightly increased the number of mosquitoes captured compared to unbaited traps. Based on the modest increase in mosquito collection for traps paired with the oxalic acid, it is not a suitable stand-in for either of the 2 traditional CO_2 sources. Conversely, the yeast-generated CO_2 resulted in collections with mosquito abundance and species richness more closely resembling those of the traditional CO_2 sources, despite achieving a lower CO_2 flow rate. Therefore, if dry ice or compressed gas cannot be acquired for vector surveillance, yeast-generated CO_2 can significantly improve trap capability.

KEY WORDS Vector surveillance, BG-Sentinel™, Centers for Disease Control and Prevention light trap, sugar-fermenting yeast, electrolyzed oxalic acid

INTRODUCTION

Successful vector-borne disease control requires accurate surveillance data. Without knowledge of an area's arthropod vectors and their densities, control efforts may not be successful. Traps are integral to mosquito surveillance programs, as they directly provide the data to estimate mosquito density and diversity, population age-structure, and pathogen infection rates (Garrett-Jones 1964, Reisen and Pfuntner 1987, Gu et al. 2003, Kilpatrick et al. 2005, Eisen and Eisen 2008). Population data from trap surveillance provide key information for the development of disease risk assessment models (Diuk-Wasser et al. 2006), and the implementation of mosquito control measures (Amoo et al. 2008). While most traps deployed for mosquito surveillance are capable of attracting mosquitoes without an olfactant, the addition of a CO_2 source greatly improves the trap's surveillance capability (Carestia and Savage 1967, Vythilingam et al. 1992). Dry ice and compressed CO_2 gas from canisters are commonly employed as baits for mosquito surveillance, with each source significantly augmenting the efficacy of the traps they are paired with (Newhouse et al. 1966, Carestia and Horner 1968). For military purposes, CO_2 canisters and dry ice for vector surveillance can be difficult to acquire during contingency operations in remote regions (AFPMB 2002, 2013), especially if continuous supplies are needed to sustain a long-term surveillance program. For this reason, alternative and easily acquired sources of CO_2 are required during contingency operations. Due to the nature of military operations, alternative CO_2 generators should be lightweight, durable, portable, easy to use, repair, and maintain, and be deployable for long periods. Most of these requirements would also make alternative CO_2 sources useful for public health programs throughout the developing world, where dry ice and compressed gas are unobtainable or too expensive (Oli et al. 2005, Moncaz et al. 2013).

The risk of vector-borne disease transmission in an environment is dependent on both the species in an area and their population density (Olson et al. 1979, Eldridge 2004, Scott and Morrison 2004). Consequently, for a novel source of CO_2 to be a useful stand-in for surveillance when dry ice and compressed CO_2 are unavailable, it must attract the same species of mosquitoes as traditional CO_2 sources, at comparable densities, and also attract species that unbaited traps may fail to collect. In this study, the ability of 2 novel sources of CO_2 to meet these requirements was assessed during day and night

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14. ABSTRACT
Carbon dioxide (CO2) sources improve the efficacy of mosquito traps. However, traditional CO2 sources (dry ice or compressed gas) may be difficult to acquire for vector surveillance during military contingency operations. For this reason, a new and convenient source of CO2 is required. Two novel CO2 generators were evaluated in order to address this capability gap: 1) an electrolyzer that converts solid oxalic acid into CO2 gas, and 2) CO2 produced by yeast as it metabolizes sugar. The flow rate and CO2 concentration produced by each generator were measured, and each generator???s ability to attract mosquitoes to BG-SentinelTM traps during day surveillance and to Centers for Disease Control and Prevention light traps with incandescent bulbs during night surveillance was compared to dry ice and compressed gas in Jacksonville, FL. The electrolyzed oxalic acid only slightly increased the number of mosquitoes captured compared to unbaited traps. Based on the modest increase in mosquito collection for traps paired with the oxalic acid, it is not a suitable stand-in for either of the 2 traditional CO2 sources. Conversely, the yeastgenerated CO2 resulted in collections with mosquito abundance and species richness more closely resembling those of the traditional CO2 sources, despite achieving a lower CO2 flow rate. Therefore, if dry ice or compressed gas cannot be acquired for vector surveillance, yeast-generated CO2 can significantly improve trap capability.

15. SUBJECT TERMS

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surveillance. One novel source of CO_2 was generated by a fermentation chamber, in which yeast metabolized sucrose. This source had been shown to attract various mosquito species in field and laboratory conditions (Saitoh et al. 2004, Oli et al. 2005, Smallengange et al. 2010, Obenauer et al. 2013, Steiger et al. 2014), but had not been implemented in the surveillance of day-feeding mosquitoes or compared to other novel sources of CO_2. The other novel source involves a chemical reaction between water, oxalic acid, and an electrical current to generate CO_2. To our knowledge, this technology has not yet been field tested or compared to other sources of CO_2.

MATERIALS AND METHODS

CO_2 sources

Four CO_2 sources were evaluated and compared in this study. Dry ice and pressurized CO_2 gas from a cylindrical tank are conventional sources of CO_2 gas, and were compared to the 2 novel CO_2 generators. For the appropriate treatments, approximately 2 kg of dry ice was placed in a thermos to provide CO_2 gas for the duration of the 6-h and 12-h trapping periods. The dry ice was expected to release 0.55 m^3 CO_2/kg (Caldwell el al. 2006), and sublimate in 24 h at an average flow rate of 763.88 ml/min. This estimation provides a reference point for the expected amount of CO_2 produced by dry ice, which would also be influenced by the environmental conditions in the surveillance area, and is only used to demonstrate that dry ice is releasing CO_2 gas at higher flow rates than all other sources being evaluated. Carbon dioxide gas was also supplied from 1 20-lb CO_2 tank (Praxair Inc., Danburg, CT). A gauge was placed on the valve to maintain a flow rate of approximately 250 ml/min during the surveillance periods.

The 2 novel CO_2 sources evaluated were yeast-fermenting sugar and electro-stripping a carboxylated organic compound (oxalic acid). The yeast-produced CO_2 was generated from a mixture of 35 g of Red Star® Active Dry Yeast (LeSaffre Yeast Corporation, Milwaukee, WI) and 250 g of refined sugar diluted into 2.5 liters of tap water, which was expected to produce CO_2 at approximately 220 ml/min (Smallengange et al. 2010). The mixture was hand-shaken in a 3-liter plastic bottle until the yeast appeared thoroughly mixed with the water and sugar (30–45 sec). A polyethylene tube (0.5-cm inner diam) was inserted through the lid of each bottle (Fig. 1A). The connection between the tubing and the lid was made airtight by applying outdoor caulking. Each yeast generator was tested to be airtight by creating a sealed vacuum through blocking the tube opening and squeezing the bottle. It was considered airtight if the bottle did not release air. This airtight seal of each generator was tested before each trapping period. The CO_2 generated by electro-stripping oxalic acid was produced by a Moustiq-Air™ CO_2 generator (Med-e-Cell, San Diego, CA) powered with a lithium iron (Li-Fe) battery (Tenergy Corporation, Fremont, CA). The Moustiq-Air is an electrochemical generator that electro-strips CO_2 from oxalic acid. To operate, approximately 500 ml of water are added to the reservoir holding the oxalic acid feed-ring, and a direct current power source is connected to the 2 terminals that power the electrolyzer. A 0.5-cm inner diam polyethylene tube is inserted through the gas exhaust port in the lid of the unit, allowing CO_2 delivery (Fig. 1B). The Moustiq-Air was claimed by the manufacturer to generate 12 liters of CO_2 per hour, at a flow rate of 200 ml/min. The functionality of Moustiq-Air was tested before and after each trap period by submerging the open end of the tubing under water and observing for gas bubbles.

Flow rates

Under laboratory conditions (25.9 $\pm$ 0.01°C, RH 52.1 $\pm$ 2.0%), the flow rates of the oxalic acid and yeast CO_2 sources were measured using an inverted beaker filled with water and held at the waterline in a tub of water, just above the output of the polyethylene tubing that delivered the gas from the generator. The gas that each generator produced displaced water from inside the beaker, so the amount of displaced water (ml) in 1 min was the observed flow rate (ml/min). The 1st measurement began 15 min after the yeast and sugar slurry was mixed, and subsequent recordings were made hourly over 6 h. The measurements were replicated twice each day, over the course of 4 days ($n = 8$). In addition to measuring flow rate, the concentration of CO_2, measured in parts per million (ppm), in the atmosphere was measured 30 cm from the polyethylene tube outputs of the Moustiq-Air and the yeast generator using an EasyView® 80 CO_2 Analyzer (Extech Instruments, Nashua, NH) and was compared to the atmospheric concentration of CO_2 30 cm from a thermos containing dry ice every 1 min over a 6-h period.

Study areas

Day trapping occurred in a residential neighborhood of Jacksonville, FL. Approximately 70% of the selected property was bordered by a natural area composed primarily of native, naturalized, and ornamental vegetation prone to flooding that creates ephemeral pools of standing water. Day sampling was conducted using BG-Sentinel™ (BGS) (BioGents, Regensburg, Germany), which is designed to attract *Aedes aegypti*

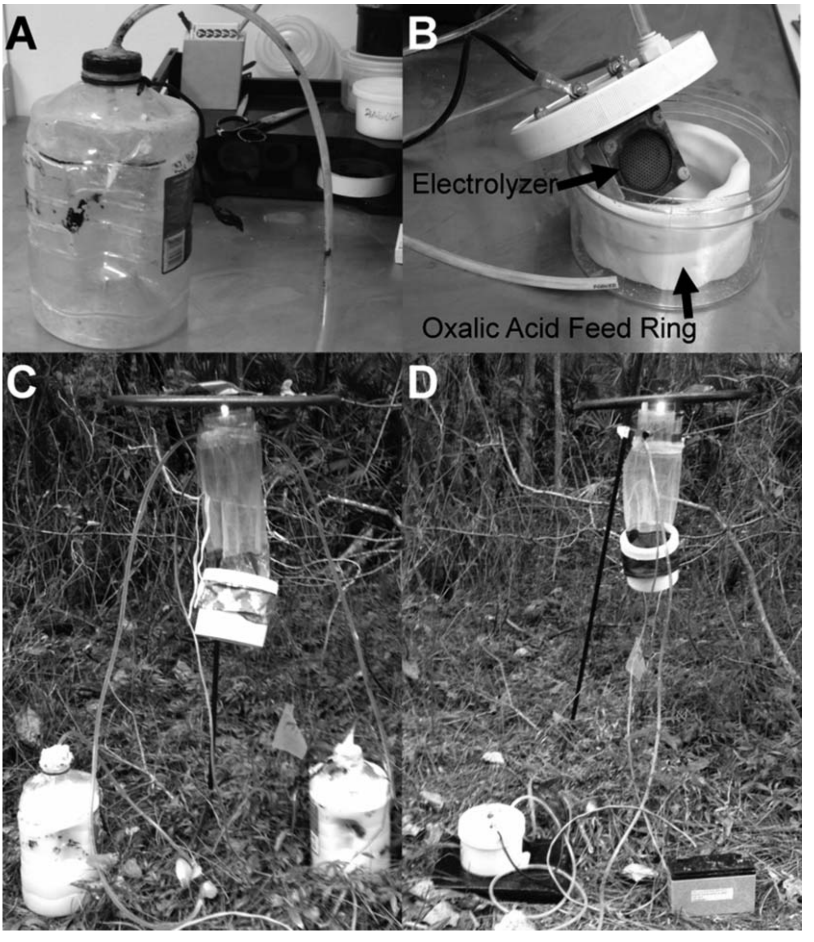

Fig. 1. Images of the (A) yeast generator, (B) Moustiq-Air™ oxalic acid generator in the laboratory, and the positioning of (C) the 2 yeast tanks and (D) the Moustiq-Air when paired with a Centers for Disease Control and Prevention light trap for night surveillance.

(Linnaeus) and *Ae. albopictus* (Skuse) (Williams et al. 2006, Kawada et al. 2007). The BGS has been observed to be more attractive to *Ae. albopictus* than other traps (Farajollahi et al. 2009). While the trap can also be used with an attractant that mimics human odors, we only evaluated the traps with the CO_2 attractants. The BGS traps were placed along the property's perimeter, spaced 20 m apart to avoid the host cues from the different CO_2 sources from mixing (Gillies and Wilkes 1970). Day trapping involved 4 treatments paired with the BGS: 1) no CO_2 source, in which the trap was placed without being baited; 2) CO_2 from dry ice, where 2 kg of dry ice were placed in an insulated thermos with ventilation holes and hung 0.5 m above the BGS; 3) CO_2 from yeast-fermenting sugar, where 2 3-liter bottles containing the yeast/sugar mixture provided CO_2 through polyethylene tubing placed near the trap's opening; and 4) CO_2 from electrolyzed oxalic acid, generated by the Moustiq-Air powered by a 40-Ah Li-Fe battery, delivered to the opening of the BGS with polyethylene tubing. A 4 × 4 Latin square design was implemented over 8 days, so that each of the 4 CO_2 sources was placed at each of the 4 locations for 2 days of trapping. The Moustiq-Air could only operate for 6 h when the 40-Ah battery was powering it; therefore, day surveillance was conducted from 0800–1400 h.

Night trapping was conducted at Naval Air Station (NAS) Jacksonville, FL, in an undeveloped area near an antenna placement. The area was part of a greater nature preserve at the air station, characterized by native vegetation, with a canopy composed primarily of slash pine (*Pinus elliottii* Engelmann) and an understory dominated by saw palmetto (*Serenoa repens* W. Bartram). The area is frequently flooded following rains, providing pools of standing water for larval mosquito habitat. The Centers for Disease Control and Prevention (CDC) light traps with

incandescent bulbs were implemented during the night surveillance since the traps are designed to sample night-feeding mosquitoes that are attracted to light. The light traps were hung approximately 1.5 m above the ground and spaced 20 m apart (Gillies and Wilkes 1970). The CDC light traps were deployed overnight for 12 h from 1830–0630 h. Five CO_2 sources were evaluated during the night sampling: 1) no CO_2 source, in which the CDC light trap was placed without being baited; 2) CO_2 from dry ice, where 2 kg of dry ice was placed in an insulated thermos with ventilation holes cut into the bottom and hung 0.25 m above the trap; 3) CO_2 gas from a 20-lb CO_2 canister (Praxair Inc.) delivered at approximately 250 ml/min by a polyethylene tube to approximately 5 cm from the trap entrance; 4) CO_2 from yeast-fermenting sugar, where 2 3-liter fermentation bottles delivered CO_2 to the trap via polyethylene tubing to the trap entrance (Fig. 1C); and 5) CO_2 from electrolyzed oxalic acid generated by the Moustiq-Air powered by an 80-Ah Li-Fe battery, delivered via polyethylene tubing to the trap entrance (Fig. 1D). A 5 $\times$ 5 Latin square design was implemented over 20 nights, so that each treatment was placed at each of the 5 trap locations 4 times. All mosquitoes collected during the sampling periods were identified to species with taxonomic keys. Nightly temperature and rainfall were recorded during the study. The surveillance data set for both day and night surveillance periods consisted of mosquito catches (abundance) and number of species present (species richness).

Statistical methods

All statistical tests were performed in Intel® Visual Fortran Composer XE 2013 (Intel Corporation, Santa Clara, CA) with $\alpha = 0.05$. In a preliminary analysis with goodness-of-fit tests, the Kolmogorov–Smirnov test (Smirnov 1939) showed that the data sets were nonnormal, and the Bartlett test (Bartlett 1937a, 1937b) showed nonhomoscedastic behavior (nonhomogeneity of variances). Hence, nonparametric Kruskal–Wallis (K-W) hypothesis tests (Kruskal and Wallis 1952) were conducted in this study.

A 2-way K-W hypothesis test was utilized to assess differences in CO_2 flow rates (ml/min) between the yeast- and oxalic acid–generated CO_2 at the 7 observation times (hour 0–6). A nonparametric 1-way K-W test assessed the effect of the surveillance date on mosquito catch rate (number of individuals) and richness (number of species). A 2-way K-W test assessed differences in catch rate and species richness among treatments, among positions, and the treatment $\times$ position interaction. Additional 2-way K-W tests were also conducted to determine how each treatment, the trap placement, and the interaction of the treatment and location affected the number of *Anopheles* spp. and *Ae. albopictus* captured during the night and the day surveillance, respectively. These species were selected for individual analysis due to their ability to transmit malaria and dengue virus, respectively. Tukey multiple-comparisons tests were conducted to identify the specific variables that were significantly different from each other for each K-W test (Zar 1999).

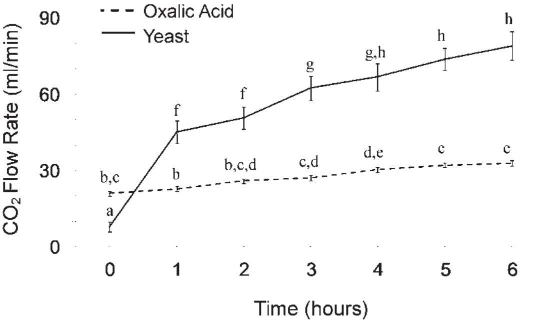

Fig. 2. Average flow rate (ml/min) $\pm$ S_0 generated by a single yeast-fermentation bottle (35 g yeast, 250 g sugar, 2.5 liters water), and by the Moustiq-Air™ electro-stripping CO_2 from oxalic acid, recorded hourly. Averages presented with different letters are significantly different from each other, as determined through Tukey post hoc analysis.

RESULTS

A single yeast-generator bottle was found to generate gas at a greater rate (ml/min) than the electrolyzed oxalic acid ($\chi^2 = 37.38$, df = 1, $P < 0.0001$). The flow rate produced by the electrolyzed oxalic acid was calculated to be on average 27.45 $\pm$ 1.72 ml/min, with the flow rate never exceeding 33 ml/min during the 7 h of operation, whereas a single yeast-fermentation bottle generated an average flow rate of 55.15 $\pm$ 9.08 ml/min, and a maximum average flow rate of 79.2 ml/min. Overall, the flow rate of both generators increased with time ($\chi^2 = 6.04$, df = 6, $P < 0.0001$), and the yeast generator's output increased at a faster rate than the oxalic acid ($\chi^2 = 2.63$, df = 6, $P = 0.02$) (Fig. 2).

The baseline atmospheric CO_2 concentration recorded in the laboratory by the EasyView 80 CO_2 Analyzer was measured at 477.96 $\pm$ 1.65 ppm during a 6-h period. The average concentration of CO_2 measured 30 cm from the output of the electrolyzed oxalic acid during 6 h of operation was 874.27 $\pm$ 2.74 ppm. The average concentration of CO_2 measured 30 cm from the output of a single yeast fermentation tank over 6 h was 2,365.54 $\pm$ 46.38 ppm. The average concentration of CO_2 dry ice released into the atmosphere 30 cm from the ventilations holes of the insolated thermos could not be measured with the equipment we had available. The CO_2 analyzer

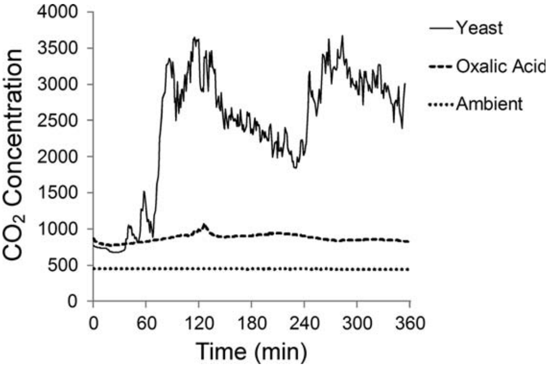

Fig. 3. Concentration of CO_2 (ppm) measured 30 cm from the output of a single yeast-fermentation bottle (35 g yeast, 250 g sugar, 2.5 liters water), and by the Moustiq-Air™ electro-stripping CO_2 from oxalic acid, recorded every 1 min over the course of 6 h using an EasyView® 80 CO_2 Analyzer under laboratory conditions compared to the ambient CO_2 levels recorded in the same space when no CO_2 source was present. The concentration of dry ice cannot be presented since the concentration 30 cm from its output overloaded the CO_2 analyzer. The CO_2 concentration in the atmosphere near dry ice is expected to be >6,000 ppm during the entire observation time period.

had a maximum detection limit of 6,000 ppm, which the dry ice exceeded during the 6-h observation. Therefore, the average CO_2 concentration of the atmosphere 30 cm from the dry ice was >6,000 ppm. The time-specific concentration of CO_2 generated by each source is presented in Fig. 3.

Results of the nonparametric K-W test demonstrated mosquito abundance and species richness were not affected by the date of collection during the day surveillance. However, the date that the night surveys were conducted significantly influenced the mosquito abundance (χ^2 = 36.11, df = 19, P = 0.01), but not species richness. Tukey groupings showed a general increase in the mosquito abundance over time, until abundance peaked (August 28) and then declined (Fig. 4).

A total of 21 species of mosquito, in 9 genera, were collected between the day and night surveys (Table 1). Results of the nonparametric K-W tests showed that, for both the BGS and the CDC light trap, trap location did not influence mosquito abundance or species richness. Additionally, no significant treatments × position interactions were observed for the day or night trapping on mosquito abundance or species richness.

For the BGS trap, the treatment type influenced the number of mosquitoes trapped (χ^2 = 4.57, df = 3, P = 0.009) and the number of species attracted (χ^2 = 5.72, df = 3, P = 0.003). Tukey post hoc tests demonstrated that the addition of a CO_2 source to the BGS resulted in a greater number of mosquitoes collected compared with traps that lacked CO_2. Dry ice outperformed the 2 novel sources of CO_2, while the yeast outperformed the oxalic acid (Fig. 5A, 5C).

The treatment type also significantly influenced the number of mosquitoes trapped (χ^2 = 12.18, df = 4, P < 0.0001) and the number of species collected (χ^2 = 9.98, df = 4, P < 0.0001) by the CDC light traps. Tukey post hoc tests demonstrated that the addition of a CO_2 source significantly increases the number of mosquitoes a CDC light trap attracts, with dry ice outperforming all other sources, while the yeast outperformed the oxalic acid but not the CO_2 tank (Fig. 5B). Additionally, the CO_2 sources increased the number of species that are attracted to the light trap, with the dry ice attracting the greatest number of species, followed by the CO_2 tank. The CO_2 generated from yeast was found to attract more species of mosquitoes than the CO_2 from oxalic acid, and both yeast- and oxalic acid–generated CO_2 outperformed unbaited traps (Fig. 5D).

The location of the trap within each study area did not influence the number of *Anopheles* spp. collected during night surveillance or the number of *Ae. albopictus* collected during the day surveillance. Additionally, no significant interactions were observed between treatment type and location during the night and day surveillance of the *Anopheles* spp. or *Ae. albopictus*, respectively. There was an effect of the treatment on the number of *Anopheles* spp. collected (χ^2 = 10.9779, df = 4, P < 0.0001), where each CO_2 source increased the number of *Anopheles* spp. sampled by baited traps compared to the unbaited control, with dry ice providing the greatest improvement, followed by the CO_2 tank, then yeast, and finally oxalic acid (Table 2). However, when species of *Anopheles* were independently evaluated the treatment was only found to affect the number of *An. crucians* (Wiedemann) (χ^2 = 8.32, df = 4, P < 0.0001) attracted by each trap, while the addition of a CO_2 source had no effect on the number of *An. quadrimaculatus* (Say) collected (Table 2). Finally, the treatment type did not affect the number of *Ae. albopictus* collected by the BGS traps during the day surveillance (Table 2).

DISCUSSION

Since there was no effect of location on the traps' surveillance efficacy, the mosquito populations at each site appeared homogeneously distributed throughout each environment. The lack of an effect of trap date at the day trapping location, in suburban Duval County, FL, suggests the mosquito populations did not fluctuate during the study, nor did the species

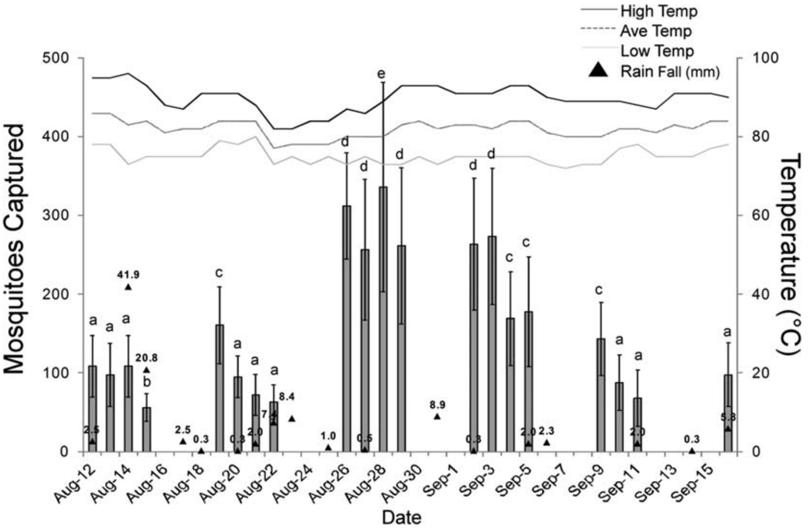

Fig. 4. Bar plots showing average numbers of mosquitoes ($\pm$ S_0) captured each night by Centers for Disease Control and Prevention light traps from August 12, 2013, to September 16, 2013, at Naval Air Station Jacksonville, FL. Different letters above the date represent significant differences between the average number of mosquitoes collected in each trap, as determined through a Tukey post hoc analysis. The environmental conditions recorded at the field site during the study are also represented. Black triangles represent dates that rain was recorded (mm).

composition in the area change in response to weather patterns or from our sampling efforts. In contrast, the mosquito populations from the natural area of NAS Jacksonville, FL, showed a strong dependence on time. This was likely an effect of the local environmental conditions, since the mosquito populations appeared to have increased following rains that flooded the area.

Table 1. Total number of each mosquito species collected during day surveillance with BG-Sentinel™ traps, over 8 days (0800–1400 h) and during night surveillance with Centers for Disease Control and Prevention light traps, over 20 nights (1830–0630 h) when baited with CO_2 generated by dry ice, a pressurized CO_2 tank (Tank), a yeast and sugar solution (Yeast), and electro-stripped oxalic acid (OA).

	Day					Night					
Species	Dry ice	Yeast	OA	None	Total	Dry ice	Tank	Yeast	OA	None	Total
Aedes albopictus	18	19	11	4	52	2	2	0	1	0	5
Ae. atlanticus	6	1	0	0	7	3,042	2,416	2,011	616	520	8,605
Ae. sollicitans	0	0	0	0	0	4	1	0	0	0	5
Ae. triseriatus	0	0	0	0	0	9	2	0	0	1	12
Ae. fulvuspallens	0	0	0	0	0	13	12	14	4	1	44
Ae. infirmatus	20	1	1	0	22	533	741	218	85	25	1,602
Anopheles crucians	0	0	0	0	0	1,069	833	537	300	253	2,992
An. quadramaculatus	0	0	0	0	0	101	194	93	38	35	461
Coquillettidia perturbans	0	0	0	0	0	25	21	4	9	1	60
Culex erraticus	0	0	0	0	0	17	3	0	0	0	20
Cx. nigripalpus	2	0	0	0	2	220	69	70	29	18	406
Cx. quinquefasciatus	0	0	0	0	0	3	0	0	0	0	3
Cx. restuans	0	0	0	0	0	24	7	12	5	3	51
Culiseta melanura	0	0	0	0	0	0	1	0	0	0	1
Mansonia dyari	0	0	0	0	0	6	6	4	0	0	16
Ms. titillans	0	1	0	1	2	0	0	0	0	0	0
Psorophora ciliata	0	0	0	0	0	21	15	10	8	5	59
Ps. columbiae	0	0	0	0	0	156	135	47	22	15	375
Ps. ferox	25	4	0	0	29	194	170	55	12	2	433
Uranotaenia lowii	0	0	0	0	0	12	7	5	5	4	33
Ur. sapphirina	0	0	0	0	0	21	16	40	18	33	128
Total	71	26	12	5	114	5,472	4,651	3,120	1,152	916	15,311

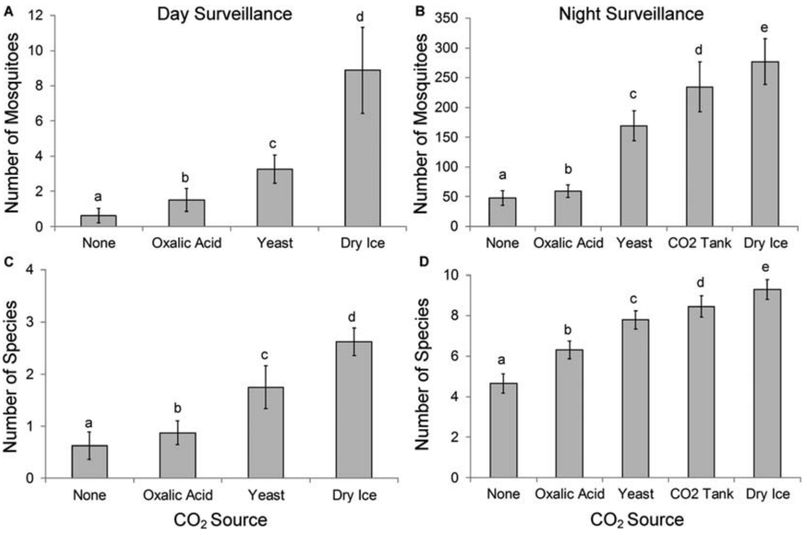

Fig. 5. (A, B) Average number of mosquitoes (abundance) ($\pm S_0$) and (C, D) average number of species (species richness) ($\pm S_0$) captured by (A, C) BG-Sentinel™ traps or (B, D) the Centers for Disease Control and Prevention light trap when baited by different CO_2 sources, during day and night surveillance, respectively. The letters above each average represents the means that are statistically different, as determined through a Tukey post hoc analysis.

As expected, the addition of any CO_2, regardless of its source, improved the BGS and the CDC light trap's ability to collect mosquitoes (Carestia and Savage 1967, Vythilingam et al. 1992). Nevertheless, dry ice outperformed all other CO_2 sources, resulting in a 13.5-fold increase in the average number of mosquitoes collected relative to unbaited BGS traps and a 5.9-fold increase relative to unbaited CDC light traps. Since the attractiveness of CO_2-baited traps is positively correlated with CO_2 flow rate (Carestia and Horner 1968), the high rate of CO_2 release during dry ice sublimation is likely the cause of the drastic improvement for both traps' surveillance ability when baited with dry ice compared to the other treatments. The dry ice also yielded the greatest species richness, providing a more accurate picture of mosquito diversity in an environment compared with other CO_2 sources.

The CO_2 generated from electro-stripped oxalic acid increased the number of mosquitoes captured by 1.9-fold compared to the unbaited BGS during day surveillance and by 1.2-fold compared to unbaited CDC light traps during night surveillance. However, during day and night surveillance it resulted in very low catches compared to all other CO_2 sources. Additionally, the flow rate in which the oxalic acid produced CO_2 was about 10% of what the manufacturer claimed it should generate. The observed flow rate of the oxalic acid was also substantially less than that of the yeast-fermented CO_2. Therefore, it is likely the low numbers of mosquitoes attracted by the oxalic acid is due to the low flow rate achieved during this evaluation.

The CO_2 produced by the yeast-fermenting sugar outperformed the other novel CO_2 source, but did not compare favorably to the 2 traditional

Table 2. Average number of *Aedes albopictus* ± SE collected during day surveillance with BG-Sentinel™ traps, over 8 days (0800–1400 h), and average number of *Anopheles* spp. ± SE collected during night surveillance with Centers for Disease Control and Prevention light traps, over 20 nights (1830–0630 h) when baited with different sources of CO_2. The letters next to each value represent the groupings that resulted from the Tukey multiple-comparisons test conducted on the results of the nonparametric Kruskal–Wallis tests, which assessed effects of the CO_2 source on mosquito collections. Different letters designate differences among treatments within each column.

	Day surveillance	Night surveillance	
Source	*Ae. albopictus*	*An. crucians*	*An. quadramaculatus*
CO_2 tank	—	41.65 ± 6.93 a	5.05 ± 1.66 a
Dry ice	2.25 ± 0.56 a	53.45 ± 8.13 b	9.70 ± 3.76 a
Yeast	2.38 ± 0.63 a	28.26 ± 4.06 c	4.90 ± 2.06 a
Oxalic acid	1.38 ± 0.60 a	15.00 ± 2.17 d	1.90 ± 0.63 a
None	0.50 ± 0.33 a	12.65 ± 2.56 e	1.75 ± 0.66 a

sources. Despite the lower catch rate, the yeast-generated CO_2 is expected to provide a useful alternative when dry ice and CO_2 tanks are unavailable, since it improved the total number of mosquitoes captured by the BGS trap by 3.3-fold during day surveillance and the CDC light trap by 3.6-fold during night surveillance. It also attracted the relatively common species in the environment that were also collected by traps baited with dry ice. The mosquito species not collected by traps baited with yeast-generated CO_2 were rarely captured by the other CO_2 sources, so those not attracted by yeast are expected to occur in the environment at such low densities that they would pose a minimal threat to public health (Olson et al. 1979, Scott and Morrison 2004). The flow rate achieved by the yeast mixture in this study did not match what was observed by Smallengange et al. (2010) during field trials, even though the same ratio of yeast and sugar was evaluated here. The yeast used in this study may have influenced this, as could have the different environmental conditions under which each was tested. However, we produced similar flow rates to what Steiger et al. (2014) produced using a similar ratio of sugar and yeast, under similar laboratory conditions. Despite inconsistencies reported among these studies (Smallengange et al. 2010, Steiger et al. 2014), it is important to note that even at flow rates less than that expected from dry ice, the yeast generator still attracts a strong representation of the mosquito species in the environment at rates that are expected to provide adequate information to determine which vectors are present in an area. Moreover, the simplicity and speed that CO_2 can be generated with yeast, sugar, and water in a plastic bottle has advantages for fieldwork in remote localities. Therefore, in the absence of other CO_2 sources, yeast-generated CO_2 can be expected to provide a reliable alternative for surveillance.

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